A highly-sensitive rapid sandwich immunoassay for human fetuin A using the one-step antibody immobilization procedure

Sandeep Kumar Vashist[1], Chandra Kumar Dixit[2], Felix von Stetten[1,3], Roland Zengerle[1,3,4]*

[1] HSG-IMIT - Institut für Mikro- und Informationstechnik, Georges-Koehler-Allee 103, 79110 Freiburg, Germany.

[2] Centre for Bioanalytical Sciences, National Centre for Sensor Research, Dublin City University, Glasnevin, Dublin, Ireland.

[3] Laboratory for MEMS Applications, IMTEK – Department of Microsystems Engineering, University of Freiburg, Georges-Koehler-Allee 103, 79110 Freiburg, Germany.

[4] BIOSS – Centre for Biological Signalling Studies, University of Freiburg, 79110 Freiburg, Germany.

*Corresponding author's e-mail: sandeep.kumar.vashist@hsg-imit.de

A highly-sensitive rapid sandwich immunoassay (IA) was developed for the detection of human fetuin A (HFA), which is a specific biomarker for hepatocellular carcinoma and atherosclerosis, and associated with arthritis, cardiovascular diseases, malaria, diabetes and metabolism-associated syndrome. It employs the one-step antibody (Ab) immobilization procedure[1], where the anti-HFA Ab, mixed with 1% (v/v) 3-aminopropyltriethoxysilane (APTES) in the ratio of 1:1 (v/v), were dispensed to 96-well microtiter plate (MTP) wells and left incubated for 30 min, which leads to the leach-proof binding of capture anti-HFA Ab to the MTP (Fig. 1A). The developed IA has significantly reduced overall immunoassay duration, many-fold higher sensitivity, reduced complexity and lower cost than the conventional and our previously-developed IA procedures[2-6] (Fig. 1B). It detects HFA in the dynamic range of 4.9-20,000 pg mL^{-1} with the limit of detection and analytical sensitivity of 7 pg mL^{-1} and 10 pg mL^{-1}. The intra- and inter-day variability were 1.2-8.5 and 2.1-10.2, respectively, while the EC_{50} was 2.6 ng mL^{-1}. The developed IA had no interference with the immunological reagents (Fig. 1C) and correlated well with the commercial kit. It detects HFA concentrations spiked in complex patient sample matrices i.e. diluted whole blood and plasma (Fig. 1D). The Ab-bound MTPs, stored at 4ºC in 0.1 M phosphate-buffered saline (PBS), pH 7.4, were found to be highly stable as there was no decrease in their functional activity even after 6 weeks. Therefore, the developed IA can be reliably employed in the clinical, industrial and other bioanalytical settings. It has tremendous potential for the development of highly-sensitive *in vitro* diagnostic kits and biosensors for numerous disease biomarkers and analytes.

References:

1. Vashist, S.K., Czilwik, G., Roth, G., Buderer, J., 2012. European Patent Appl. No. EP13172022.9.
2. Vashist S.K., 2013. Biosens. Bioelectron. 40, 297-302.
3. Dixit, C.K., Vashist, S.K., MacCraith, B.D., O'Kennedy, R., 2011. Nat. Protoc. 6, 439-445.
4. Dixit, C.K., Vashist, S.K., O'Neill, F.T., O'Reilly, B., MacCraith, B.D., O'Kennedy, R., 2011. Anal. Chem. 82, 7049-7052.
5. Vashist, S.K., 2012. Diagnostics 2, 23-33.
6. Vashist, S.K., Dixit, C.K., MacCraith, B.D., O'Kennedy, R., 2011. Analyst 136, 4431-6.

(A)

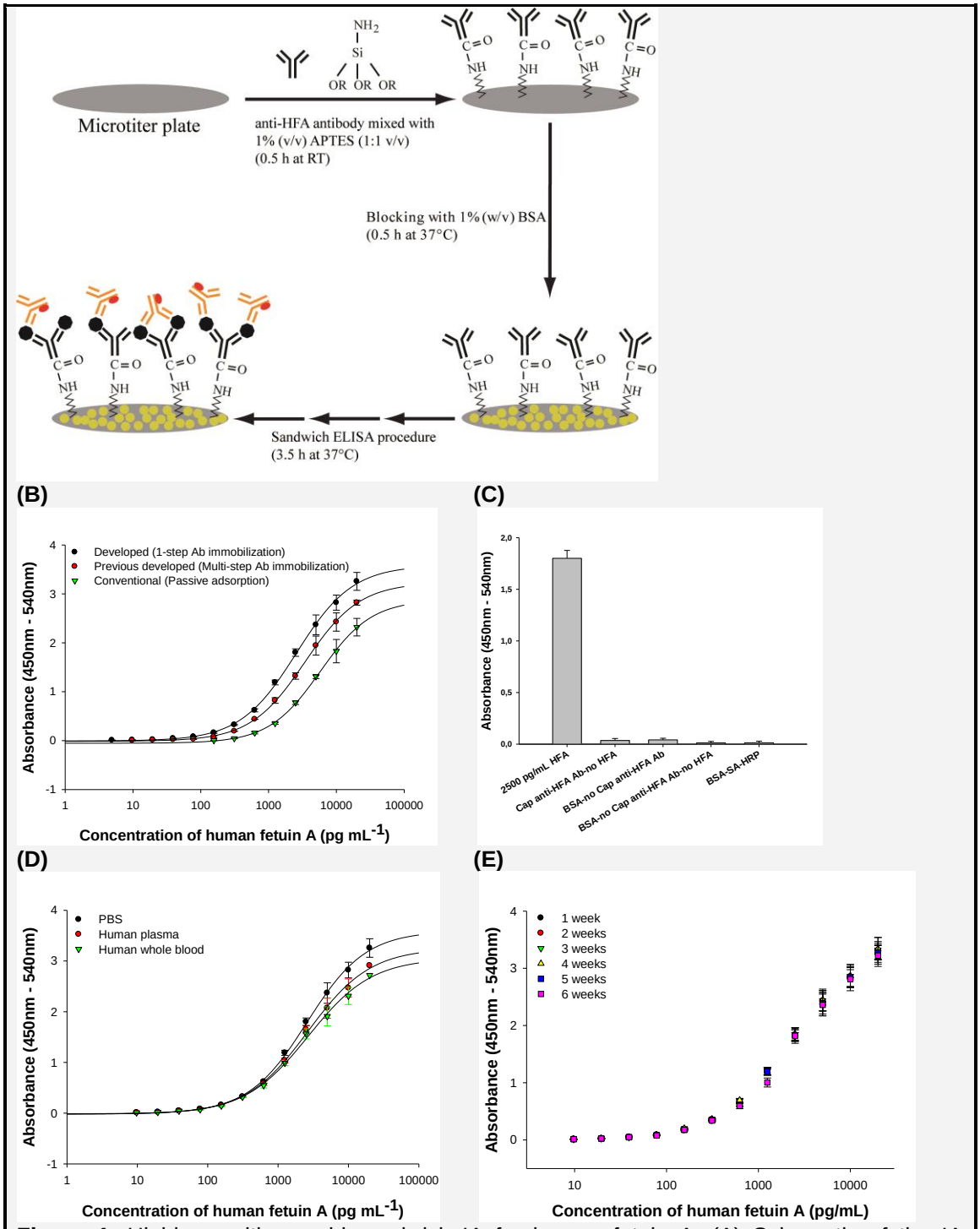

Figure 1. Highly-sensitive rapid sandwich IA for human fetuin A. (A) Schematic of the IA procedure, (B) Comparison of the developed IA with our previously developed and commercial IA procedures, (C) Experimental process controls, (D) Detection of HFA concentrations spiked in diluted human plasma and whole blood, and (E) Stability of anti-HFA Ab-bound MTPs (stored at 4ºC in 0.1 M phosphate-buffered saline (PBS), pH 7.4).